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Association study of *APC* polymorphisms with colorectal cancer in Han Chinese

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ABSTRACT

Objectives: Colorectal cancer (CRC) is a common malignancy with worldwide prevalence. Familial adenomatous polyposis (FAP), a predisposition syndrome of CRC, is caused by germ line mutations in the *APC* gene. Mutations in *APC* are thought to be an early event in colorectal tumorigenesis. We hypothesized that common variants in *APC* might be associated with CRC.

Design and methods: A case-control study genotyping ten SNPs was conducted in 312 CRC patients and 270 normal controls in the Chinese Han population.

Results: The genotype frequency of rs2019720 showed a significant difference between cases and controls ($p = 0.046$, after Bonferroni correction). For the three pairs of SNPs in strong LD, we carried out haplotype analyses but no significant association was detected.

Conclusion: Our results suggest that *APC* polymorphisms might be associated with CRC in the Chinese Han population.

Introduction

Colorectal cancer (CRC) is a common cancer and is also one of the leading causes of cancer-related death worldwide [1]. In Asia the incidence of CRC has been on the increase in recent decades [2]. Most cases of CRC occur sporadically whereas approximately 5% of the cases are due to the inherited genetic alterations [3], suggesting the combined contribution of genes and environment. In order to allow effective cancer prevention programs, intensive studies into the development of CRC and tumor progression are imperative.

Familial adenomatous polyposis (FAP), a model of CRC development, is inherited as an autosomal dominant disease [4]. The adenomatous polyposis coli (*APC*) gene, a tumor suppressor gene, plays a key role in FAP [5]. In about 80% of individuals suffering from this syndrome, a germ line mutation can be observed in the *APC* gene [6]. This mutation leads to the development of thousands of colorectal adenomas and the initiation of the event for sporadic colorectal tumorigenesis [7]. Mutations in *APC* are also identified in approximately 18% of somatic breast cancers [8]. Approximately 25% of breast cancer patients suffer loss of heterozygosity (LOH) at the *APC* locus [8].

The *APC* gene is located at chromosome 5q21–q22, encoding a large protein which has multiple functional domains. Previous research has confirmed that APC provides protection against carcinogenesis [9,10]. A major clue came from the finding that the APC protein was associated with β-catenin [11]. Studies revealed that β-catenin interacted with transcriptional factors belonging to the TCF/LEF family, activating the transcription of many target genes such as C-MYC, Cyclin D1, BMP4 and CD44 [12]. The discovery that APC regulates β-catenin levels after its re-introduction into CRC cells suggests that APC is involved in the regulation of cell growth [13]. Moreover, APC functions at enhancers to directly repress Wnt target genes through a complex with the β-TrCP protein, in competition with β-catenin-activating complexes [14].

While APC acts as an antagonist in the Wnt-signaling pathway, this protein is also believed to affect cell adhesion, cell migration and

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chromosome stability. The C-terminus of APC has been found to interact with the microtubule binding protein EB1, mediating the stabilization of microtubules [15]. Loss of APC function is suspected to contribute to chromosome instability, through the altering of chromosome segregation at low levels [12]. APC regulation of such cell processes implicates its role in tumor formation. Although germ line mutations in the *APC* gene are found only in the settings of FAP and attenuated FAP [16], it is possible that variants in the *APC* gene are related to CRC, most (~95%) of which are found in non-hereditary cases.

To date, very few studies have focused on the *APC* polymorphisms in Chinese subjects. The present study was designed to investigate the association between common genetic variants in the *APC* gene and an increased risk of CRC. We examined ten single nucleotide polymorphisms (SNPs) of the *APC* gene in CRC patients compared with healthy controls in the Chinese Han population.

Material and methods

Subjects

The case group used in this study was composed of 312 sporadic CRC patients (178 males and 134 females, age: 61.23 ± 14.03 years). Each of these patients has no family history of CRC, i.e. none of their sibling, mother or father has suffered from CRC. The control group consisted of 270 healthy controls (145 males and 125 females, age: 43.53 ± 7.94 years). All subjects recruited were of Han Chinese origin. All the CRC patients had been treated with curative resection between 1999 and 2007 at the surgical department of Shanghai First People's Hospital or Shanxi People's Hospital, China. The pathologic tumor staging was performed according to Duke's criteria. Informed consent was obtained from all participants for the genetic analysis. The study protocol was reviewed and approved by the ethics committee of the Human Genetics Center in Shanghai. DNA extraction was carried out according to standard procedures with phenol/chloroform purification.

Genotyping

The genetic polymorphisms we examined include four intronic SNPs (rs2019720, rs6594646, rs4705486, rs2464803) and six exonic SNPs (rs2229992, rs351771, rs41115, rs42427, rs459552, rs465899) which had been reported by Chen et al. [17]. SNPs were selected from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) to cover a region of about 102.9 kb. All ten SNPs were genotyped using the TaqMan® assay method on the ABI 7900 DNA detection system (Applied Biosystems, Foster City, CA). Probes and primers were designed by the Assay-on-Design service of Applied Biosystems. The standard PCR was performed using the Taqman® Universal PCR Master Mix (Applied Biosystems) reagent. Cycle parameters were 10-min, 95 °C step required to activate AmpliTaq Gold enzyme, followed by 45 cycles with 15 s at 95 °C denaturation and 1 min at 60 °C annealing/extension.

Statistical analysis

We used SHEsis (http://202.120.31.177/myanalysis.php) to analyze Hardy–Weinberg equilibrium, allelic and genotypic distributions and pairwise linkage disequilibrium [18]. This online software integrates efficient analysis tools for case-control studies and implements a Monte Carlo simulation strategy [19]. The discrepancies of allele and genotype frequency between CRC patients and controls were compared using a χ^2 test. Linkage disequilibrium (LD) of all pairs of SNPs was estimated with D' as the standardized measurement. Odds ratios (ORs) and their 95% confidence intervals (CIs) were also calculated. Haplotype reconstruction was initially performed on the Haploview program [20] and further analysis was carried out on SHEsis. For all analyses, p values

Table 1
The distributions of genotypes and alleles for the ten SNPs in the *APC* gene.

SNP (location)	Genotype frequency			χ^2	p value*	Allele frequency		χ^2	p value*	Odds ratio (95%CI)
rs2019720 (intron)	AA	AG	GG			A	G			
CRC	29(0.128)	77(0.339)	121(0.533)	10.774	**0.0046**	135(0.297)	319(0.703)	3.512	0.061	1.29 (0.99–1.69)
NZ	29(0.112)	126(0.485)	105(0.404)			184(0.354)	336(0.646)			
rs6594646 (intron)	AA	AG	GG			A	G			
CRC	29(0.105)	102(0.371)	144(0.524)	6.163	**0.046**	160(0.291)	390(0.709)	3.105	0.078	1.26 (0.97–1.63)
NZ	27(0.105)	122(0.473)	109(0.422)			176(0.341)	340(0.659)			
rs4705486 (intron)	GG	GT	TT			G	T			
CRC	138(0.517)	101(0.378)	28(0.105)	3.119	0.210	377(0.706)	157(0.294)	2.217	0.136	0.82 (0.63–1.06)
NZ	116(0.441)	117(0.445)	30(0.114)			349(0.663)	177(0.337)			
rs2464803 (intron)	CC	CT	TT			C	T			
CRC	141(0.483)	117(0.401)	34(0.116)	1.209	0.546	399(0.683)	185(0.317)	0.404	0.525	1.08 (0.84–1.39)
NZ	119(0.442)	120(0.446)	30(0.112)			358(0.665)	180(0.335)			
rs2229992 (exon)	CC	CT	TT			C	T			
CRC	132(0.520)	91(0.358)	31(0.122)	4.519	0.104	355(0.699)	153(0.301)	1.645	0.200	0.84 (0.65–1.09)
NZ	115(0.437)	118(0.449)	30(0.114)			348(0.662)	178(0.338)			
rs351771 (exon)	CC	CT	TT			C	T			
CRC	13(0.048)	71(0.263)	186(0.689)	3.016	0.221	97(0.180)	443(0.820)	0.203	0.652	0.93 (0.68–1.27)
NZ	8(0.030)	86(0.321)	174(0.649)			102(0.190)	434(0.810)			
rs41115 (exon)	CC	CT	TT			C	T			
CRC	6(0.029)	50(0.243)	150(0.728)	2.376	0.305	62(0.150)	350(0.850)	2.144	0.143	0.77 (0.54–1.09)
NZ	9(0.035)	77(0.303)	168(0.661)			95(0.187)	413(0.813)			
rs42427 (exon)	AA	AG	GG			A	G			
CRC	189(0.730)	62(0.239)	8(0.031)	2.907	0.234	440(0.849)	78(0.151)	2.394	0.122	0.77 (0.56–1.07)
NZ	172(0.662)	79(0.304)	9(0.035)			423(0.813)	97(0.187)			
rs459552 (exon)	AA	AT	TT			A	T			
CRC	2(0.008)	33(0.124)	231(0.868)	1.924	0.382	37(0.070)	495(0.930)	1.648	0.199	0.75 (0.48–1.17)
NZ	2(0.008)	43(0.167)	213(0.826)			47(0.091)	469(0.909)			
rs465899 (exon)	CC	CT	TT			C	T			
CRC	11(0.038)	77(0.269)	198(0.692)	1.485	0.476	99(0.173)	473(0.827)	0.154	0.695	0.94 (0.69–1.28)
NZ	7(0.027)	80(0.310)	171(0.663)			94(0.182)	422(0.818)			

*Pearson's p value, significant p (<0.05) values are in boldface, CRC: colorectal cancer, NZ: normal control.

Table 2
Estimation of linkage disequilibrium of the ten SNPs.

	rs2019720	rs6594646	rs4705486	rs2464803	rs2229992	rs351771	rs41115	rs42427	rs459552	rs465899
rs2019720	–	0.995	0.863	0.921	0.917	0.735	0.901	0.882	0.948	0.768
rs6594646	0.961	–	0.873	0.901	0.899	0.745	0.895	0.860	0.952	0.735
rs4705486	0.727	0.738	–	0.799	0.777	0.771	0.746	0.717	0.976	0.753
rs2464803	0.825	0.808	0.616	–	0.945	0.876	1.000	1.000	0.953	0.881
rs2229992	0.820	0.801	0.578	0.885	–	0.776	0.909	0.917	0.780	0.760
rs351771	0.249	0.268	0.293	0.362	0.296	–	0.861	0.880	1.000	1.000
rs41115	0.335	0.344	0.245	0.434	0.363	0.697	–	0.984	0.852	0.867
rs42427	0.316	0.315	0.231	0.429	0.371	0.717	0.968	–	0.792	0.878
rs459552	0.162	0.175	0.180	0.165	0.111	0.386	0.314	0.270	–	0.965
rs465899	0.255	0.256	0.271	0.360	0.278	0.967	0.728	0.718	0.360	–

D' values are shown above and r^2 values below the diagonal.

were two tailed and the significance level was set at $p<0.05$, after Bonferroni correction.

Results

A total of 312 CRC patients and 270 controls were included in our study. The allele and genotype frequencies of the ten SNPs are listed in Table 1. The observed genotype distributions did not show any significant deviations from Hardy–Weinberg equilibrium in the control subjects (data not shown). For SNP rs2019720, statistically significant difference in genotype frequency was detected between cases and controls ($p=0.0046$, $p=0.046$, after Bonferroni correction). SNP rs6594646 showed a slight difference in genotype frequency before correction ($p=0.046$, $p=0.46$, after Bonferroni correction). There were no significant associations for any of the other genetic polymorphisms.

For each pairwise combination of the SNPs, we calculated D' and r^2 in all the subjects as the metric of LD (shown in Table 2). Three pairs of SNPs were revealed to be in strong LD (rs2019720-rs6594646, rs41115-rs42427 and rs459552-rs465899). We therefore constructed two-locus haplotypes with these combinations of markers (shown in Table 3). Those haplotypes with an estimated frequency of less than 3% in both case and control groups were excluded from analysis. Globally, no positive association with CRC was found for these haplotypes ($p=0.112$, $p=0.114$, $p=0.415$, respectively). Haplotype analysis combining all ten SNPs showed no significant differences (global $p=0.250$).

Discussion

To explore the association of SNPs in the *APC* gene with CRC, we conducted a case-control study in 582 Han Chinese subjects. In the single-site tests for association, SNP rs2019720 appeared to be associated with CRC risk. We observed a significant difference in genotype frequency ($p=0.0046$, $p=0.046$, after Bonferroni correction). The genotype GG was more frequent in patients than in healthy controls (53.3% versus 40.4%), suggesting that it might be a risk genotype for CRC. The genotype AG of rs2019720 also contributed to the association (33.9% in cases versus 48.5% in controls), and might therefore be a protective factor against CRC. However, no significant haplotype associations were found.

Table 3
Estimated *APC* haplotype frequencies and association significance.

Haplotype[a]	Case frequency	Control frequency	χ^2	*p* value	OR (95%CI)
Group 1: M1, M2					
GG	0.701	0.654	2.522	0.112	1.25 (0.95–1.65)
AA	0.290	0.338	2.522	0.112	0.80 (0.61–1.05)
Global				0.112	
Group 2: M7,M8					
CG	0.144	0.186	2.503	0.114	0.75 (0.52–1.07)
TA	0.845	0.814	2.503	0.114	1.34 (0.93–1.92)
Global				0.114	
Group 3: M9, M10					
AC	0.063	0.085	1.714	0.190	0.73 (0.46–1.17)
TC	0.105	0.099	0.112	0.737	1.07 (0.72–1.61)
TT	0.828	0.816	0.402	0.526	1.11 (0.80–1.53)
Global				0.415	

M1: rs2019720, M2: rs6594646, M7: rs41115, M8: rs42427, M9: rs459552, M10: rs465899.
[a] Haplotypes with a frequency under 3% were excluded.

In the past few years, genome-wide association studies (GWAS) have identified several susceptibility loci for CRC [21]. Each variant has a relatively small effect on CRC risk and a large part of the genetic contribution remains unknown [22]. Furthermore, high-penetrance genes including *APC* and the DNA mismatch repair (MMR) genes are responsible only for the familial CRC syndromes [21]. Investigators have proposed different models to explain the pathogeny of CRC. The risk genotype found in our sample was quite common and therefore consistent with the "common disease, common variant" hypothesis [23].

The *APC* gene is 138.7 kb in size and composed of 15 exons. Ten SNPs were examined in this study covering most of the APC region. Four (rs2019720, rs6594646, rs4705486 and rs2464803) of the selected markers were located in noncoding introns. Although lacking in possible coding or transcriptional activity, our data showed intronic SNP rs2019720 to be a susceptibility locus. It can be implied that rs2019720, probably together with other genetic variants, might somehow be connected with functional effects that contribute to disease formation. Among the other six exonic SNPs genotyped, rs459552 is a missense variant while the remaining five (rs2229992, rs351771, rs41115, rs42427 and rs465899) are synonymous polymorphic sites. In a previous investigation including all the six markers, Chen at el. demonstrated significant associations at rs2229992, rs41115 and rs459552 in Taiwanese subjects [17]. Compared to the study by Chen et al., our sample was much larger and thus had a lower probability of false-positive results.

Most of the research evidence to date has centered on four *APC* missense variants at codon 1307, 1317, 1822 and 2502 [16]. In FAP patients, germ line mutations close to codon 1300 correlate with LOH as the second 'hit' [24]. Protein-truncating *APC* changes tend to occur within a particular region between codon 1250 and 1450 which is termed the mutation cluster region (MCR) [25]. The APC protein contains seven 20-amino acid repeats essential for β-catenin regulation. Mutations near codon 1300 yield a truncated protein product with only one 20-amino acid repeat, whereas other mutations in the MCR result in varying numbers of subsequent repeats [12]. Codon 1822 lies near the fifth β-catenin binding repeat [17]. SNP rs459552 at codon 1822 has been the most common missense polymorphism [17,26–30]. In recent years, investigators have focused on the interaction between rs459552 and lifestyle factors in colorectal adenoma risk [16,31–34]. Both dietary fat intake and postmenopausal hormone therapy (HRT) have been reported as potential risk factors for colorectal adenoma or CRC. More research work will

be needed to analyze the correlation between genetic and environmental factors associated with CRC.

Conclusion

In summary, our results indicate that SNP rs2019720 of *APC* might be correlated with CRC in the Chinese Han population. This finding needs to be tested in bigger samples and in other ethnic groups. To clarify whether common *APC* variants are related to CRC susceptibility, genetic analyses with more saturated SNP coverage of the region are necessary. The data we obtained may provide a reference for future studies on the role of *APC* in the etiology of CRC.

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